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Pachyphloeus depressus, a new green truffle from China

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Abstract — *Pachyphloeus depressus* sp. nov. is described based on specimens collected from the valley of the Jinshajiang River in southwest China. The species can be distinguished from all other species in *Pachyphloeus* by its smooth, greenish brown ascomata covered with yellow-green hairs, and its globose ascospores with spines that have somewhat T-shaped or capitate apices. Phylogenetic analyses of the ITS and ITS-LSU supported the erection of a new species.

Key words — morphology, phylogeny, *Pezizaceae*, taxonomy

Introduction

During recent investigations of truffles in southwestern China, some small green truffles were collected from Qiaojia County, Yunnan Province, and Huili County, Sichuan Province, in the soil of mixed forest with *Pinus armandii* Franch. as the dominant species. Both counties are near the Jinshajiang River. According to the local people, these green truffles are not uncommon in these areas, although they are not found in large quantities. The species appears to be easily confused with the Chinese black truffle *Tuber pseudohimalayense* G. Moreno et al. (= *T. pseudoexcavatum* Y. Wang et al.) because the green truffles also have a distinctive depression at the base of the ascomata, somewhat similar to the excavated ascomata of *T. pseudohimalayense*. Macroscopically, our collections led us to *Pachyphloeus* Tul & C. Tul. [nom. illeg.; = *Pachyphlodes* Zobel; *Pezizaceae*], a green truffle genus that is well known from both Europe and North America (Calonge et al. 2002, Colgan & Trappe 2004, Frank et al. 2006, Fogel & States 2002, Gilkey 1954, Healy et al. 2009, Lange 1956, Moreno-Arroyo et al. 1996, Pegler et al. 1993). After critical comparison of the morphological and molecular characters of our collections with the known

species in both genera, we are confident that our collections represent an undescribed species.

Materials & methods

Morphological observation

The specimens were collected from Sichuan and Yunnan Provinces of China and deposited in the Herbarium of the Biology Department, Capital Normal University, Beijing, China (BJTC). Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 3% KOH; Melzer's reagent (Dring 1971: 98), or 0.1% (w/v) cotton blue in lactic acid. For scanning electron microscopy (SEM), ascospores were scraped from the dried gleba onto doubled-sided tape, which was mounted directly on an SEM stub, coated with platinum-palladium, and examined and photographed using a HITACHI S-4800 scanning electron microscope.

Phylogenetic studies

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube, together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was extracted using the PeqLab E.Z.N.A. Fungal DNA Kit following the manufacturer's protocol. The internal transcribed spacer ribosomal RNA gene (ITS) and the 28S large subunit ribosomal RNA gene (LSU) were amplified using the universal fungal primers ITS1/ITS4 and LROR/LR5 (White et al. 1990, Vilgalys & Hester 1990). PCRs were performed in 50 µl reactions containing 2 µl of DNA template, 2 µl of primer (10 µM/L), and 25 µl of 2× Master Mix (Tiangen Biotech (Beijing) Co. Ltd.). PCR reactions were run as follows: an initial denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 2 min, 55°C for 25 s, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd., Beijing, China, for purification, sequencing, and editing. The other ITS and LSU sequences included in this study were downloaded from GenBank. GenBank accession numbers are shown in Table 1.

DNA sequences were aligned using Clustal X (Thompson et al. 1997). The alignment was manually adjusted using Se-Al v.2.03a (Rambaut 2000). Phylogenetic analysis was conducted separately on the ITS and ITS-LSU alignments. Each aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). Maximum parsimony analysis was conducted using heuristic searches with 1,000 replicates of random-addition sequence, and the tree-bisection-reconnection (TBR) branch-swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed. Outgroups in these analyses were sequences from *Peziza infossa* Fogel & States and *Peziza* cf. *badioconfusa* Korf, chosen following our preliminary data on phylogenetic relationships across the genus *Pachyphloeus* (Læssøe & Hansen 2007, Healy et al. 2009)

Table 1 Specimens and sequences used in this study.
New sequences are set in bold font.

Species	Voucher	Accession number in GenBank	
		ITS	LSU
Pachyella clypeata (Schwein.) Le Gal	–	–	EU543195
Pachyphloeus austro-oregonensis J.L. Frank & Trappe	SOC 775	AY830854	–
	SOC: 775 (isotype)	JX414191	–
P. carneus Harkn.	RH00	EU543199	EU543199
	RH25	EU543202	EU543202
	JT12818	EU543208	EU543208
P. citrinus	JRWL2497	EU543196	EU543196
	MIN: RH840	JN409343	JN409343
P. conglomeratus	17057	JF908512	–
	MA: 29354	JN102487	–
P. depressus	BJTC FAN302 (holotype)	**KP027405**	**KT220750**
	BJTC FAN324	**KP027406**	**KT220751**
P. marroninus Healy et al.	RH299	EU427549	EU427549
	Garcia3557	EU427551	EU427551
	JT32454	EU543209	EU543209
P. melanoxanthus (Berk.) Tul. & C. Tul.	MM1860	EU543194	EU543194
	–	JF908511	–
P. thysellii	JT13182	EU543197	EU543197
P. virescens	RH279	EU543198	EU543198
Peziza infossa	–	DQ974817	DQ974817
Peziza cf. *badioconfusa*	RH7	EU571229	EU571229

Results

Molecular phylogenetics

In our ITS analyses, the sequences of the new species, *Pachyphloeus depressus*, clustered as an independent clade with bootstrap support of 100% (Fig. 1). The MP tree of combined ITS-LSU (Fig. 2) revealed that the sequences of *P. depressus* were similarly grouped in a clade with 100% bootstrap support. Also with strong bootstrap support was their grouping in a distinctive clade with three other *Pachyphloeus* species (*P. citrinus* Berk. & Broome known from Europe, *P. virescens* Gilkey, and *P. thysellii* W. Colgan & Trappe both from North America).

Taxonomy

Pachyphloeus depressus L. Fan, **sp. nov.** Fig. 3

MycoBank MB810700

Differs from all other *Pachyphloeus* spp. by its smooth, greenish-brown ascomata.

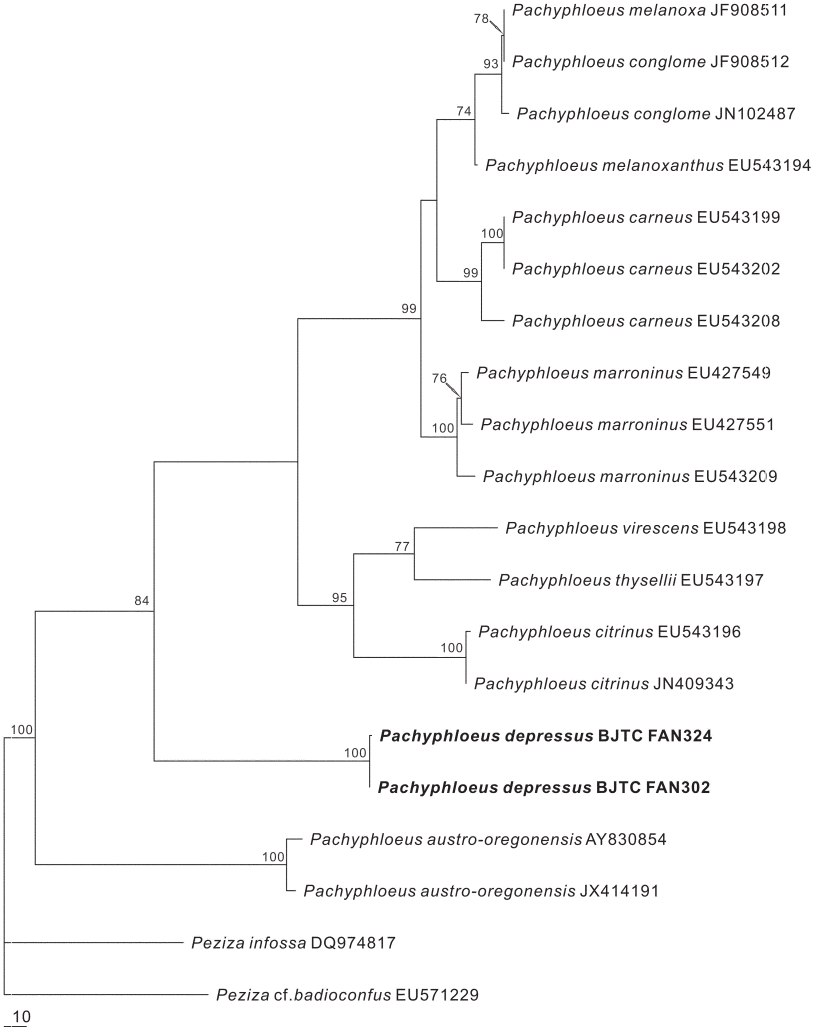

Fig. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences from *Pachyphloeus* species using two *Peziza* species as outgroup. A total of 220 characters out of 676 were parsimony-informative. Tree length (TL) = 661 steps, consistency index (CI) = 0.7655, retention index (RI) = 0.7939, homoplasy index (HI) = 0.2345, and rescaled consistency index (RC) = 0.6077. Bootstrap values of more than 70% are shown above the respective branches. Novel sequences are printed in bold.

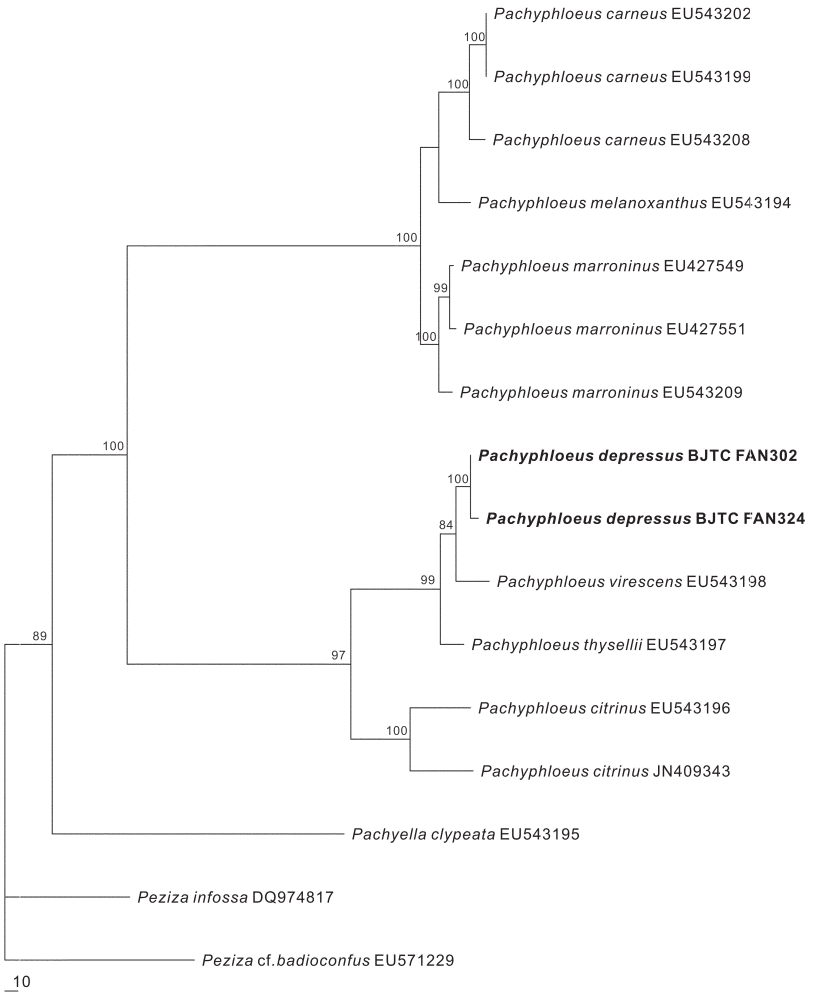

Fig. 2. Phylogeny derived from maximum parsimony analysis of the ITS-LSU rDNA sequences from *Pachyphloeus* species and *Pachyella clypeata* using two *Peziza* species as outgroup. A total of 336 characters out of 1458 were parsimony-informative. Tree length (TL) = 993 steps, consistency index (CI) = 0.8087, retention index (RI) = 0.7974, homoplasy index (HI) = 0.1913, and rescaled consistency index (RC) = 0.6449. Bootstrap values of more than 70% are shown above the respective branches. Novel sequences are printed in bold.

Type: China. Yunnan Province, Qiaojia County, in soil under forest dominated by *Pinus armandii*, 16 Oct. 2013, Jin-zhong Cao 807 (**Holotype**, BJTC FAN302; GenBank KP027405, KT220750).

Etymology: *depressus* (Lat.), referring to the shape of the ascomata.

Ascomata oblate spheroid or cake-like, 0.9–2.1 cm diam., texture rubbery, smooth, yellow-green, brownish green to dark brownish green, covered with densely yellow-green hairs in places; the apical orifice more or less circular and usually distinctively depressed, dark brown and often with flat or pyramidal warts; basal tuft of hyphae earth brown, sometimes located in a distinctive superficial depression. Odor pungent when ripe, somewhat like burnt potato. Taste not tested. Peridium at maturity one layer of pseudoparenchymatous tissue, 250–500 μm thick, composed of sub-angular to rectangular cells, light brown, thin-walled, cells 10–60 μm diam., more or less arranged with long axes parallel to the outer surface; towards inside grading to a thin layer of parallel textura intricata, hyphae hyaline, 5–10 μm wide next to gleba. The outermost cells are often irregularly elongated with ends free, creating a peridial surface that is more or less felty or hairy. Gleba yellow-green to brownish green when fresh, with large and rare whitish yellow veins. Glebal hyphae 7.5–10 μm wide at septa, hyaline, thin-walled, some cells swollen to 15 μm. Asci not forming a palisade, subglobose or broadly elliptic, 70–95 × 45–75 μm, thin-walled, sessile or with a short stalk, with 8 irregularly arranged ascospores, non-reactive to Melzer's solution with pretreatment in 3% KOH. Ascospores globose, 17.5–20 μm excluding spines, hyaline at first, then light greenish, ornamented with coarse, rod-like spines 2–2.5 μm long, apices expanding to irregular T-shape or capitate under SEM.

Additional specimen examined: **CHINA**. **Sichuan Province**, Huili County, in soil under forest dominated by *Pinus armandii*, 16 Oct. 2013, Jin-zhong Cao & Li Fan 826 (BJTC FAN324; GenBank KP027406, KT220751).

Comments — The new species, *Pachyphloeus depressus*, is characterized morphologically by its combination of cake-like ascomata, smooth on the surface and covered with yellow-green hairs, subglobose asci, and ascospores densely covered with spines that have irregularly T-shaped tips. *Pachyphloeus depressus* is distinguished from the other *Pachyphloeus* species by its smooth ascomata, with the exception of *P. conglomeratus* Berk. & Broome known from Europe. *Pachyphloeus conglomeratus* also lacks warts on its ascomatal peridium, but instead has red-brown scurfy particles, and its ascomata are dark brown (Pegler et al. 1993) rather than the yellow-green or brownish green of *P. depressus*. Furthermore, the distinctively long clavate asci of *P. conglomeratus* also clearly separate it from *P. depressus*. Our ITS phylogeny (Fig. 1,2) shows

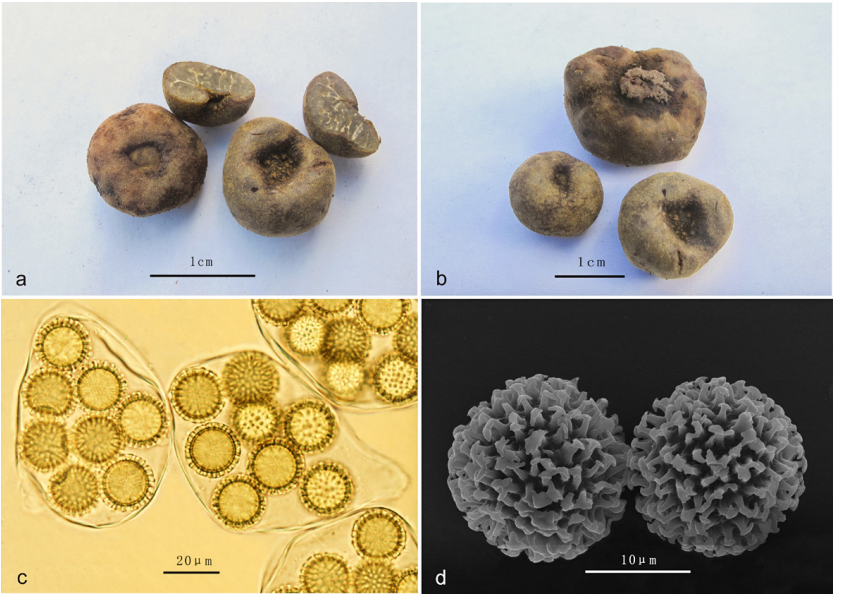

Fig. 3. *Pachyphloeus depressus* (BJTC FAN302, holotype): a,b. ascomata; c. asci and ascospores observed under the light microscope; d. ascospores observed under SEM.

that sequences of *P. depressus* group in a distinct clade with strong support (BP = 100%), further supporting the erection of a new species.

Pachyphloeus depressus and three other species in the genus cluster in a distinct clade (BP = 97%) in the tree constructed from the combined ITS-LSU database (Fig. 2). The morphological trait that these species share is ascomata that are more or less green or have yellow tints. However, *P. depressus* has ascomata that usually have flat or pyramid warts around the apical orifice only. In contrast, the ascomata of all the other three species have distinctively pyramidal warts over all their surfaces.

Pachyphloeus depressus seems not uncommon in the Jinshajiang River valley. It has been locally called the "green female truffle" because its ascomata superficially resemble that of *Tuber pseudohimalayense*, a very common truffle species in this area.

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